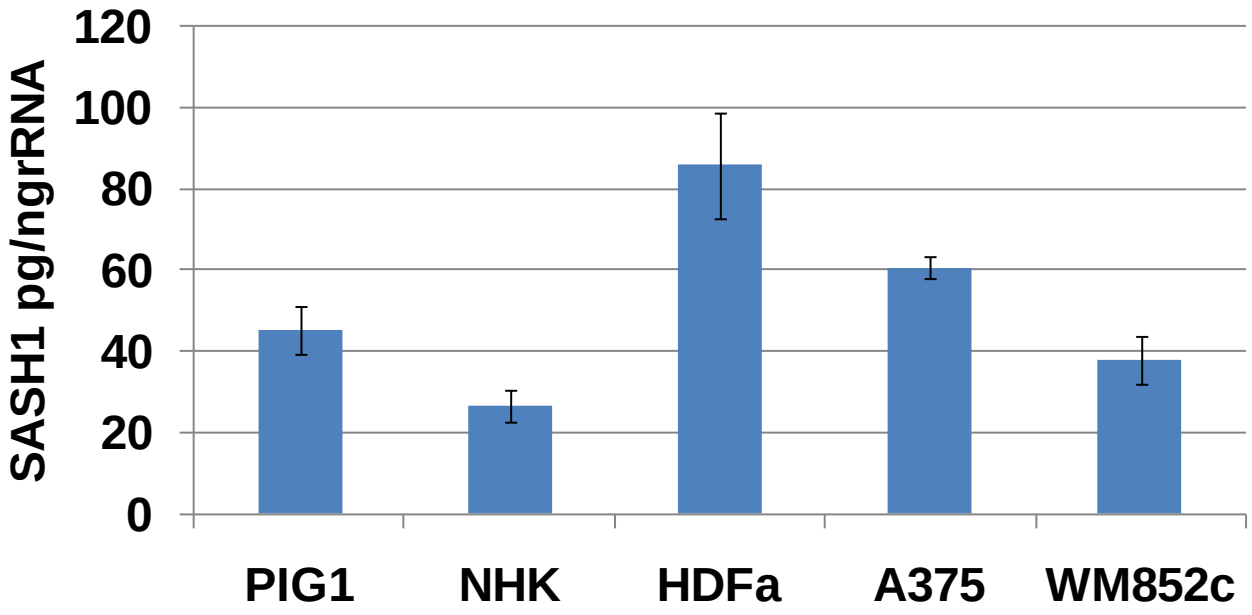

Supplemental Fig.1 SASH1 is expressed in human melanocytes, keratinocytes, fibroblasts, and melanoma cells. Various skin cells were cultured in their respective culture conditions and then harvested for total RNAs. Samples were then quantified at University of Colorado qRT-PCR CORE. PIG1 is an immortalized human melanocyte cell line, NHK is a human normal primary keratinocyte culture from Life Technologies, and HDFa is a human normal dermal fibroblast culture from Cascade biologics. A375 and WM852c are human melanoma cell lines, cultured in RPMI1640 with 10% FBS.

SUPPLEMENTAL MATERIALS AND METHODS

Subjects

All blood samples, biopsies, photographs and information were obtained after written informed patient consent were approved by the relevant institutional review boards (including permission to publish images). The diagnosis of lentigines was made independently by two dermatologists. Additional genomic DNAs were obtained from a) 150 control subjects (from the Diabetes Autoimmunity Study in the Young project) and b) 20 melanoma patients who also displayed lentigines (University of Colorado Skin Cancer Biodepository).

DNA isolation

DNA was prepared from peripheral blood samples using standard procedures or a QIAamp DNA Blood Maxi kit (Qiagen, Inc.).

Mutation detection

For all genes within the previously reported linkage analysis, primers were designed to amplify all exons and 100 bp of the intronic flanking sequences, the 5' and 3' untranslated regions, and 2000 bp of the 5' promoter region. PCR amplification of the genomic DNA was performed using standard procedures. Purified amplicons were sequenced bi-directionally using an ABI BigDye Terminator Sequencing kit and an ABI3100 Sequencer (Applied Biosystems, Culver City, California) by University of Colorado Cancer Center Sequencing Core, and analyzed by Sequencher software (Gene Codes). Sequence variants, which did not co-segregate with disease or were present in public SNP databases, were not pursued further.

Facial Pigmentation Image Capture. Images of the study subject were captured using the VISIA-Complexion Analysis (VISIA-CA) multi-modality facial imaging system according to the

manufacturer's instruction (Canfield Scientific, Inc). VISIA-CA captures regular images (standard white light) and images of melanin pigmentation (RBX®-Brown cross-polarized images), based on their RBX® technology.

Histological analysis of skin biopsies.

Four-millimeter punch biopsies from lesional (hyper-pigmented area) and adjacent non-lesional (peri-lesional area, 1 cm or more distant and normal-appearing skin) were collected from non-photoexposed region of skin on the ventral forearms. Two sections per category were harvested from each individual, and with counts resulting from an average 5 fields from each section. Biopsies from the control individuals were taken from a similar region of forearm. Flagship Biosciences processed the paraffin embedded biopsies and performed the immunohistochemistry staining with the melanocyte-specific marker, MART1. A dermatopathologist assessed the general histopathologic features.

Standard immunoflorescent staining procedures were also performed on these sections. Briefly, antigen retrieval was performed in a steamer with citrate buffer (Invitrogen). The Ki67 antibody was from Vector labs and the MART1 antibody was from Zymed. Alexa Fluor–conjugated secondary antibodies (Invitrogen) were used for secondary detection. Cells were visualized with a Nikon Eclipse 90i microscope. Quantitation of Ki67 and MART1 was performed by counting the number of positively stained cells per millimeter of tissue across the entire length of the tissue biopsy. Cells were counted independently by two individuals. To confirm our results, slides were scanned by the APERIO image analysis system. Mechanical and manual counting yielded the same results.